BRANCHING OUT
IPM NEWSLETTER FOR TREES & SHRUBS

A New York State Oak Wilt Project: a few discoveries made and lessons learned

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Information about the oak wilt disease, caused by *Ceratocystis fagacearum,* has become an increasingly more frequent topic for this newsletter and in plant disease presentations given throughout the state. Previous articles were meant to provide the *Branching Out* readership with an awareness of this devastating disease, to provide descriptions of key symptoms and to inform when identifications were made. This article will review the work involved in a recently completed Specialty Crop Block Grant project focusing on surveying for the oak wilt pathogen (with our NYS Department of Environmental Conservation [NYSDEC] collaborators) and improving the Cornell University, Plant Disease Diagnostic Clinic's (PDDC) diagnostic procedures to include adding a molecular technique.

First, a little background...

The PDDC staff identified the fungus that causes the oak wilt disease for the first time in 2008 in a small neighborhood in Schenectady County, New York. This initial finding was quite a surprise since the closest reported case of oak wilt was over 180 miles away in western Pennsylvania. The NYSDEC staff removed symptomatic and buffer red oak trees from the neighborhood in hopes of eradicating the disease. No new cases were found for five years and then in 2013, the pathogen was identified on a sample from the same neighborhood. The second identification alarmed the staff and faculty of Cornell's Plant Pathology and Plant-Microbe Biology section who questioned whether the pathogen was infecting other oaks around the state. A comprehensive project was developed and accepted through the NYS Department of Agriculture and Markets Specialty Crop Block Grant (SCBG) program. The collaborative oak wilt project, partnering with NYSDEC, was funded and performed during the growing seasons of 2015 and 2016.

Symptomatic leaves tested positive for the oak wilt pathogen © Charles Angst

Project objectives and goals

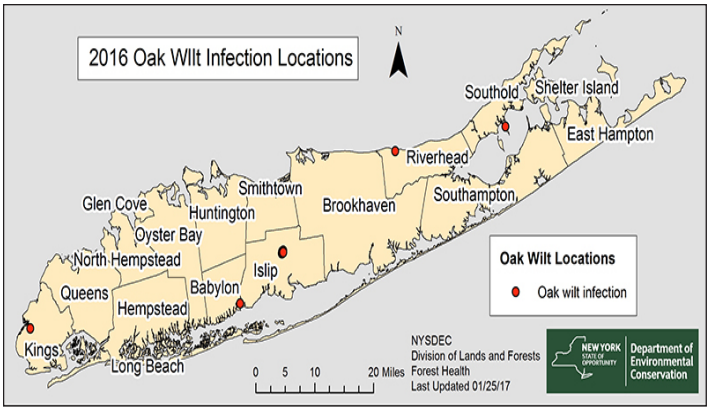
In addition to surveying for the pathogen across New York State, the project allowed the PDDC staff to implement a molecular technique and to determine if a more direct test method could reduce sample result turn-around time. The NYSDEC staff identified locations of likely candidates and collected tissue for submission. The objectives included processing the suspect samples using the traditional technique of attempting isolations from branch tissue and if characteristic fungal growth was produced, extracting DNA from the fungal isolate followed by a nested polymerase chain reaction (PCR) molecular procedure. Each sample was additionally tested by extracting DNA directly from plant tissue and processed using the nested PCR, bypassing the isolation step. Diagnostic facilities that commonly test for the oak wilt pathogen typically make isolations and identify the pathogen morphologically. When confirmation is important, the nested PCR of the fungal isolate may be performed. Because the pathogen is a very slow grower, this entire process could take more than 4–6 weeks to complete. Testing tissue directly, could reduce the diagnostic processing time significantly…from up to six weeks to a few days.

All projected goals were met for the project; 1) numerous areas across New York State were surveyed for the oak wilt pathogen and many residents and green industry members were informed to be on the lookout for the disease, 2) the presence of the pathogen was found in new locations using both morphological and molecular test methods, and 3) the direct from tissue PCR procedure was determined to be a reliable method that reduced the diagnostic process time table.

Positive samples identified

In 2015, twenty-two samples were submitted for analysis. The final sample submitted in November produced a suspect positive with the direct tissue PCR test method. Tissue from the site was re-collected and processed three times in the winter of 2016. In 2016, a total of 55 samples were submitted for analysis with 14 samples producing positive results. The overall results (for 2015 & 2106) produced fifteen 15 positive samples out of the 77 sample submissions. The positive identifications were located in four new, unique locations on Long Island, one in Brooklyn and one upstate in Canandaigua. These findings increase the total number of locations from one, the original site in Schenectady county, to seven locations where the oak wilt pathogen has been identified.

2016 oak wilt infection locations in NY state. This map does NOT include infections found in previous years. © NYS DEC



2016 Oak wilt infection locations on Long Island, NY. This map does NOT include infections found in previous years. © NYS DEC

Trenching roots of oak trees to disrupt root grafts between diseased trees and healthy trees © NYS DEC.

What did we learn from this project?

The most significant item learned was that the oak wilt pathogen was present in locations we previously were not aware of. Identifying these sites was a critically important finding. The earlier in the infection process these sites are identified, the greater chance of containing and eradicating the pathogen. This also reinforced our commitment to outreach efforts because the initial sample source was generated by alert, observant members of the green industry and community…an arborist concerned with a client's planting and a home owner and trained horticulturist that went to the Ontario County Cornell Cooperative Extension Office for help. The presentations and materials we develop on recognizing possible new or unknown pathogens and share with the public, green industry members, county office staff and others is essential training given to those we are relying on to be our eyes and ears in the field!

Another lesson learned was the fact that getting this organism in pure culture is extremely difficult. It is a weak competitor and other more robust organisms that may be secondary contaminants often outgrow the target pathogen in an artificial growth situation such as nutritive agarose plates. The pathogen can take from four to six weeks to develop the characteristics needed for a morphological identification. Cornell staff reached out to an expert from the United States Forest Service, Dr. Jennifer Juzwik. She explained that trying to isolate the pathogen from plant tissue collected during the months of November through March can be very difficult and isolating the organism during this time has an extremely low success rate.

Getting this organism in pure culture is extremely difficult.

Incorporating the molecular PCR procedure into our tools for identification worked in both situations we designed into this project. It produced results directly from the tissue samples as well as from the isolates when they were available. As mentioned above, we had problems getting some of the samples isolated that produced positive PCR results. Therefore, the PCR directly from tissue procedure we evaluated for this project identified the pathogen more often than we could confirm with isolates.

As samples were processed, the need for a second PCR procedure became obvious and would be beneficial for confirmation purposes when isolations are not productive. The second PCR procedure would also be helpful during the off season months when isolating is commonly unsuccessful. Also having this second method would help us provide more complete testing of trees that may have died the previous year and do not have living tissue available for isolation attempts. The DNA of the pathogen may remain when any living evidence is long gone.

Without the PDDC's commitment to plant disease diagnostics, the identified areas may have gone unnoticed and the potential for spread throughout NYS may have led to the significant loss of oak trees. The NYSDEC staff are committed to collecting a significant number of samples during the 2017 growing season. The PDDC staff are prepared to continue performing diagnostic procedures and has a commitment to continual improvement for not only the harmful oak wilt pathogen but any others that may invade our borders in the future. This project would not have been completed without the financial assistance of the SCBG program and the NYS Department of Agriculture and Markets. The collaboration with the NYSDEC was a vitally important aspect of the project and could not have been a success without their contributions of manpower, equipment, knowledge of tree populations and responses to positive sites.

The PDDC recently obtained a second Specialty Crop Block Grant that will allow the PDDC staff to continue improving the techniques used to identify the oak wilt pathogen. The upcoming project will begin this winter.

If you are interested in learning more and receiving various credits, consider attending an upcoming training workshop in White Plains, NY on June 13, 2017. Go to our website, www.plantclinic.cornell.edu for more information. Sample submission forms and instructions as well as links to other oak wilt related information can also be found at the site.

BOLO Alert-Be On the Look Out for:

- **Wilting leaves beginning in June, and red oaks in particular, may wilt completely in as little as 3 weeks**
- **Marginal scorch**
- **Vascular discoloration under bark**
- **Mycelial mats or pads under bark**
- **Mats/Pads have an odor of stale beer or rotting fruit**